

DETAILING LUNG CELL MORPHOLOGY IN SEPTIC PATIENTS WITH ACUTE  
RESPIRATORY DISTRESS SYNDROME

By

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### **Abstract**

With the rise of new bacterial and viral strains, curtailing invasive blood infections has become a race against the clock for hospitalized patients every day. Sepsis begins as an infection in the bloodstream. The body's immune-mediated response begins the release of cytokines targeting the pathogen. Activation of the immune system interrupts the integrity of epithelial structures in the alveoli. Without the early intervention of antibiotics, the conditions of septic patients can progress into Acute Respiratory Distress Syndrome (ARDS), jeopardizing their survival rate. Today's challenge is determining how early sepsis can be diagnosed and what changes occur in lung cells to exacerbate respiratory distress. No treatment can cure ARDS, but a combination of therapies, like mechanical ventilation and induced comas, can assist in the healing process of the lungs.

## I. INTRODUCTION

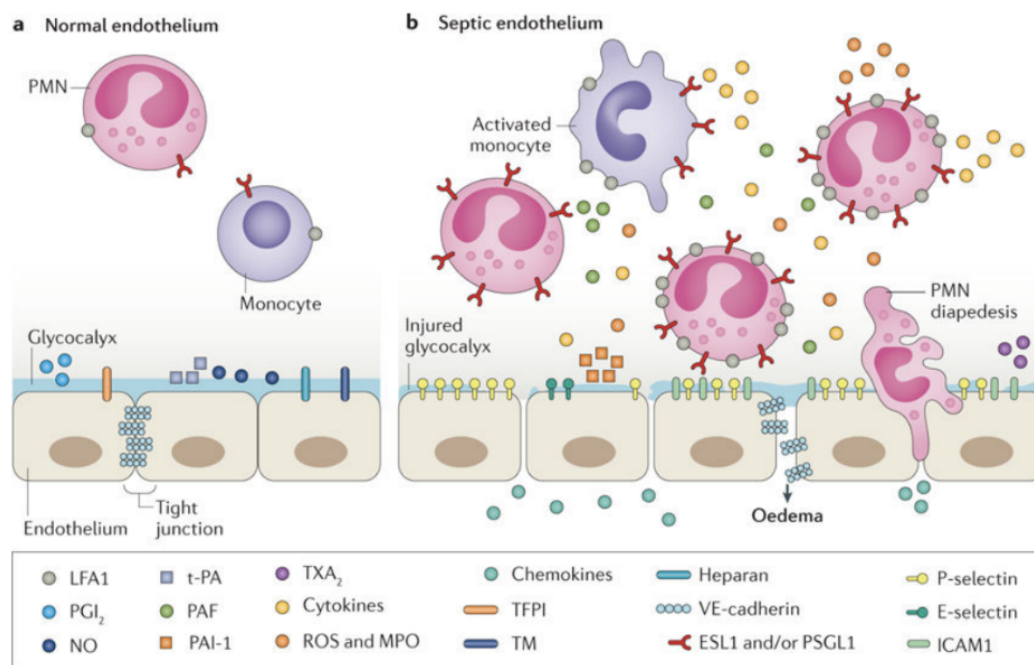
The human body can adapt to its environment, such as vasoconstriction of capillaries when temperatures drop or its ability to fight infections. What happens when a pathogen results in an overstimulation of the body's immune response? This alters homeostasis and becomes detrimental to multiple organ systems. Any bacterial or viral infection that causes the body to manifest symptoms in various systems is called sepsis (1). These manifestations can range from faulty blood pressure regulation, lactic acid buildup, poor perfusion in peripheral extremities, and, importantly, disruption of external respiration in the lungs' alveoli. Cells rely on the cardiovascular system to distribute oxygen and nutrients and remove waste products. The respiratory system regulates this distribution at gas exchange sites of thinly-walled alveoli sacs. When gas exchange does not function properly, this sets the downward path of progress for the body to maintain homeostasis. Without medical intervention, the systemic infection can cause severe sepsis, exacerbating symptoms. Patients with sepsis can go into septic shock where the body begins to decompensate for the lack of perfusion of vital organs due to hypovolemia. Since sepsis can progress differently in every patient, it is crucial for healthcare providers to identify early manifestations and begin to treat it as soon as possible.

## II. MECHANISM OF SEPSIS

In any infection, the immune response starts after the recognition of the pathogen by immune cells. Toll-like receptors (TLRs) located on the plasma membrane of dendritic cells and macrophages recognize pathogen-associated molecular patterns (PAMPs). The most commonly recognized PAMP in sepsis caused by bacteria is lipopolysaccharide, an extracellular component found in the surface membrane of bacteria. Upon recognition, immune cells begin to secrete proinflammatory cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6), and

interleukin-1 (IL-1) (1). These cytokines are responsible for systemic responses in septic patients.

The signaling pathway induced by cytokines causes changes in endothelial cell walls of blood vessels. Cytokines provoke vasodilation, disrupting the integrity of endothelial structures (Figure 1), thus triggering leakage of fluids and neutrophils in nearby tissues (2). This type of transformation in endothelial cells can damage organ function. For example, in the lungs, the tight junctions between endothelial cells form the barrier to separate the space in the alveoli from the outside. If tight junctions are separated, then communication between endothelial cells compromises the structure of the alveoli and the purpose of gas exchange (3).

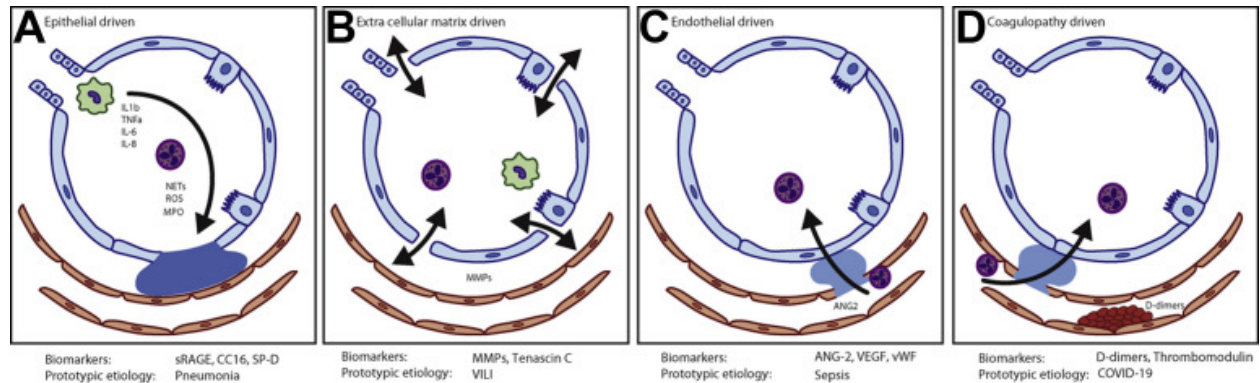


**Figure 1. Endothelium changes in sepsis.** Visual representation of the primary mechanism seen in sepsis. A. Demonstration of the vascular endothelium in its typical state. B. Activities occurring at the endothelium in septic patients. This depicts the extravasation of white blood cells and fluid due to disrupted tight junctions between endothelial cells. (Figure adapted from Hotchkiss et al., 2016).

The activation of immune cells, like dendritic cells and macrophages, can lead to the programmed death of the cell. In septic patients, the recognized pathogen is what mediates the immune response. With increased immune cell apoptosis, T-cells and dendritic cells are exhausted. No recognition of internalized pathogens by lymph organs establishes the immunocompromised state if treatment is pushed off for some time. Patients with sepsis-like symptoms benefit from early treatment to circumvent the progression of their condition.

### **III. ACUTE RESPIRATORY DISTRESS SYNDROME**

Delayed treatment sets a septic patient on a course to develop acute respiratory distress syndrome (ARDS). Sepsis is not the only cause of ARDS since conditions with direct pulmonary damage, like pneumonia, can lead to ARDS. With sepsis, the body produces a systemic response that in lung cells, it generates epithelial, endothelial, and interstitial injury (4). The alveoli fill with fluid when the endothelial integrity of lung cells is ruined in combination with the extravasation of capillaries, which compromises the lungs' function. A reduction in lung compliance increases the patient's work of breathing to compensate for their low tidal volume (5). Ultimately, gas exchange is impaired to where the circulating blood builds up carbon dioxide with low oxygen saturation, developing hypoxemia that, without intervention, results in hypoxia in peripheral tissues.



**Figure 2. ARDS-dependent changes in the alveolar unit.** Anatomical changes with their respective biomarkers induced by ARDS damage. A. Epithelial damage seen in ARDS associated with pneumonia. B. Inflammation of the extracellular matrix caused by ventilator-induced lung injury (VILI). C. Endothelial damage causing extravasation in the alveolar unit due to sepsis-induced ARDS. D. Excessive clotting in the vasculature surrounding the alveolar unit commonly seen in ARDS induced by SARS-CoV-2 infection. The production of biomarkers mitigates these changes. Such biomarkers serve as signaling promoters to create new capillaries allowing cell movement. (Figure adapted from Sinha and Bos, 2021).

The most common change alveolar units undergo in ARDS is the secretion of growth factors that disrupt the endothelium (Figure 2). Angiopoietin-2 (Ang2) and vascular endothelial growth factor (VEGF) mitigate the creation of new vasculature in the alveoli (16). Increased vasculature access into the gas exchange region allows fluid buildup, called edema. From a clinical perspective, shortness of breath and difficulty breathing are signs of compromised airway access. Long-term alveolar damage creates fibrosis, thus decreasing the volume the lungs can expand as transpulmonary pressure increases, prompting minimized lung compliance. A drop in lung compliance directly affects ventilation - a concern for doctors regarding hypoperfusion of the peripheral extremities. In conditions like these, where the work of breathing increases, advanced airway access would be considered to allow a ventilator to assist the patient in breathing.

#### IV. IMMUNE FUNCTION OF LUNG CELLS

When pathogens invade tissues, the innate immune system will respond. If the infection persists, the body generates an adaptive response within 10-14 days. Lung cells contain those same characteristics of immune surveillance. In systemic infections, like sepsis, where blood vessels tend to leak into nearby tissues, the ability to respond to infections in lung cells is crucial for the patient's survival. Patients who are immunocompromised (HIV/AIDS, cancer, etc...) have a greater risk of mortality from systemic infections that develop ARDS. Innate immune cells respond first to the infection by recruiting natural killer cells, alveolar macrophages, dendritic cells, and neutrophils.

Recruitment of innate immune cells comes at an expense for the patient. As the immune cells recognize the pathogen, they begin to secrete cytokines. Cytokines are chemical messengers that are used to stimulate and recruit other immune cells. That stimulation also includes inflammation of the surrounding tissue. Natural killer cells recognize both self and foreign cells with the help of major histocompatibility complex (MHC) molecules that reside on the cell surface. MHC molecules present pathogen peptides on the extracellular membrane to interact with other cells. With foreign cells, natural killer cells detect a foreign MHC on the surface and induce cytotoxicity through cytokine secretion (17). The activation of natural killer cells recruits dendritic cells by inducing their maturation into attacking the specific pathogen.

On the other hand, alveolar macrophages participate in innate immune activities and maintain homeostasis in the alveolar units. Alveolar macrophages attempt to limit the amount of lung inflammation by clearing the pathogen first before an adaptive response is induced (17). The benefit of alveolar macrophages residing in the alveolar lumen is their ability to renew themselves after exposure, providing some defense to immunocompromised patients; however, more invasive, systemic infections can still pose a lethal threat.

## V. PREVENTION OF ARDS

To prevent ARDS, healthcare providers need to recognize the signs and symptoms of sepsis. Once a pathogen enters the host, the body's innate immune system kicks in within minutes. This is when the clock to ARDS begins. Protocols to treat sepsis vary per hospital and their implementation depends on the patient's clinical presentation. Some hospitals fail to implement sepsis protocols contributing to patients' deaths that eventually develop ARDS (9). Sepsis protocols must be continually reviewed and refined until deaths caused by sepsis and ARDS begin to decline. The protocol (Figure 3) must dictate what a septic patient looks like in a clinical setting.

### CLINICAL PRESENTATION

When a patient's body begins to respond to a bacterial infection, innate immune cells release proinflammatory cytokines and activate transcription factors. The patient starts to have a fever, but without any antibacterial intervention, this infection becomes systemic and the body develops multiple manifestations. Preventing ARDS is synonymous with treating sepsis. At least two symptoms must be present to trigger a septic alert: temperature greater than 38°C or less than 36°C, a heart greater than 90 beats per minute, respirations greater than 20 breaths per minute, and white blood cell counts greater than 12,000 per mL or less than 4,000 per mL (2). Such signs should be followed by intravenous fluids and a two-step broad-spectrum antibiotics treatment (Figure 3). More blood work can also determine the specific pathogen in the bloodstream. However, clinical presentations vary depending on how soon the patient presents themselves for medical help.

In septic patients, the pathogen must be destroyed to prevent ARDS. The body's innate immunity begins by releasing cytokines, but that may not be enough. Patients presenting septic

symptoms should start a course of antibiotics as a precaution. Before administering antibiotics, blood cultures should be taken to allow the provider to determine what type of bacteria is in the bloodstream and if a specific antibiotic is required to treat that strain. Since providers cannot distinguish between the strains of bacteria through initial assessments, broad-spectrum antibiotics are given. Bacteria have different compositions: gram-positive and gram-negative. Differentiating between the two allows greater specificity in antibiotic treatment, which is more efficient in killing the strain. Both have certain compounds that activate the immune response such as, gram-positive bacteria containing lipoteichoic acid and gram-negative bacteria containing lipopolysaccharides (11). An antibiotic for a gram-positive bacterium might not treat a gram-negative bacterial infection because the medication cannot penetrate the polysaccharide wall. Thus, utilizing macrolides (50s ribosomal inhibitor) and fluoroquinolones (DNA gyrase inhibitors) are optimal for a broad-spectrum antibiotic treatment due to their mechanisms of blocking bacterial replication. Both result in the inhibition of proinflammatory cytokines (IL-6, IL-1 $\beta$ , TNF- $\alpha$ ) and chemokines, preventing chemotaxis of immune cells to the alveolar membrane and reducing the risk of lung injury (12). Further blood work after broad-spectrum antibiotic treatments can provide insight into whether a systemic infection persists or whether the first course of antibiotics resolved the problem.

When a provider assumes sepsis in a patient, regular blood tests can demonstrate disease progression or correction during hospital admission. No definitive test exists to diagnose sepsis or ARDS. A combination of tests and physical assessments can determine a patient's condition regarding ARDS progression. Physicians should consider pairing procalcitonin management and lung imaging to measure lung injury caused by sepsis. The precursor of calcitonin, procalcitonin, serves as a biomarker to determine sepsis progression from a bacterial infection. Proinflammatory cytokines trigger the production of procalcitonin in parafollicular cells of the thyroid glands (13). Procalcitonin levels in blood serum increase in response to a bacterial infection, not a viral infection. Utilizing procalcitonin levels daily tracks if an antibacterial

treatment is appropriate for the patient. Ideally, if the patient's systemic bacterial infection responds to antibiotics, then procalcitonin levels will decrease. Increasing procalcitonin levels correlate to severe sepsis and ARDS, thus inducing lung injury (14). Along with tracking procalcitonin serum levels, patients should undergo regular computer tomography (CT) scans of their lungs. CT scans generate a 3D view of the lungs, enabling providers to spot any attenuations of ARDS in the upper and lower lobes. Early detection of ARDS is accomplished by reporting pulmonary contusions (15) in CT lung scans. With patients in a more progressed stage of ARDS, prolonged ventilation can lead to visible fibrosis in the lobes, proving CT scans to be beneficial at any level of ARDS as opposed to the traditional bedside chest x-rays (15).

On top of assessing patients for sepsis and providing first-line treatments, identifying individuals at high risk of developing ARDS prevents their disease progression. Not all patients that enter the emergency room will display sepsis symptoms. Patients admitted into the hospital for routine procedures can develop sepsis during their stay. From a bedside perspective, providers must practice aseptic techniques to prevent the transmission of pathogens to vulnerable patients. Patients recovering from high-risk, invasive surgeries have a greater chance of becoming septic and developing ARDS (2). Potential targets exist in units where patients are in long-term recovery (such as intubations) to prevent ARDS. Mechanical ventilation, positioning, and paralysis of patients can collapse the lungs and induce an inflammatory response. This response increases alveolar epithelial and pulmonary endothelial permeability causing surfactant abnormalities, neutrophil invasion, and ARDS (2).

The presentation of sepsis varies in patients because not all symptoms are present at the assessment time. At least two abnormal clinical manifestations should trigger a vigorous, antibacterial treatment for sepsis to prevent ARDS progression. In combination with antibiotics, blood work can determine the severity of the patient's condition, which could assist the provider in ordering specific treatments for the patient. Since sepsis can also develop in hospitalized

patients, avoiding the transmission of pathogens is a way to prevent infections in high-risk patients.

## **VI. POTENTIAL THERAPIES**

Therapies for ARDS patients become complex because of the factors that come into play. The primary goal is to keep patients alive on a treatment course to recovery. By this stage, patients with ARDS are assisted by mechanical ventilation and being treated by vasopressors and antibiotics required to treat sepsis. Before any therapy can be considered, the patient must be stable to undergo such procedures. A risk of treatments becoming harmful for the patient exists due to the vulnerability of their immune systems. Sepsis-induced ARDS must be treated simultaneously while the patient undergoes therapy. If their sepsis goes out of control, the patient is at risk of dying from septic shock.

A potential treatment for ARDS patients is to recreate the lung anatomy by introducing mesenchymal stem cells or alveolar type II cells. In the experimental animal model, the rat groups will not receive both cell types, but both types produce the same effects despite their different molecular mechanisms. Alveolar type II cells currently reside in the alveolar membrane. Attempting to repair the lung anatomy in patients with impaired pulmonary function seems like an appropriate therapy. Transplanting mesenchymal stem cells in damaged lungs differentiate into alveolar type II cells, which restore their active presence of secreting surfactant and anti-inflammatory cytokines. When studied in rats with lipopolysaccharide/hydrochloric acid-induced ARDS, both cell types increased their survival rates, reduced neutrophil infiltration in the alveoli, and decreased edema by reducing permeability within 72 hours of initiating therapy (6). The notable outcomes of this therapy were the survival rates and the reduction of neutrophil infiltration. Neutrophil invasion on the

alveolar membrane is the catalyst of oxidative stress and activation of proinflammatory cytokines resulting in lung injury (7). Despite their promising results, excessive proliferation from mesenchymal stem cells can worsen pulmonary function by invading nearby tissue. Using alveolar type II cells instead of mesenchymal stem cells poses less risk since those cells are already differentiated. Still, harvesting alveolar cells is more complicated than stem cells.

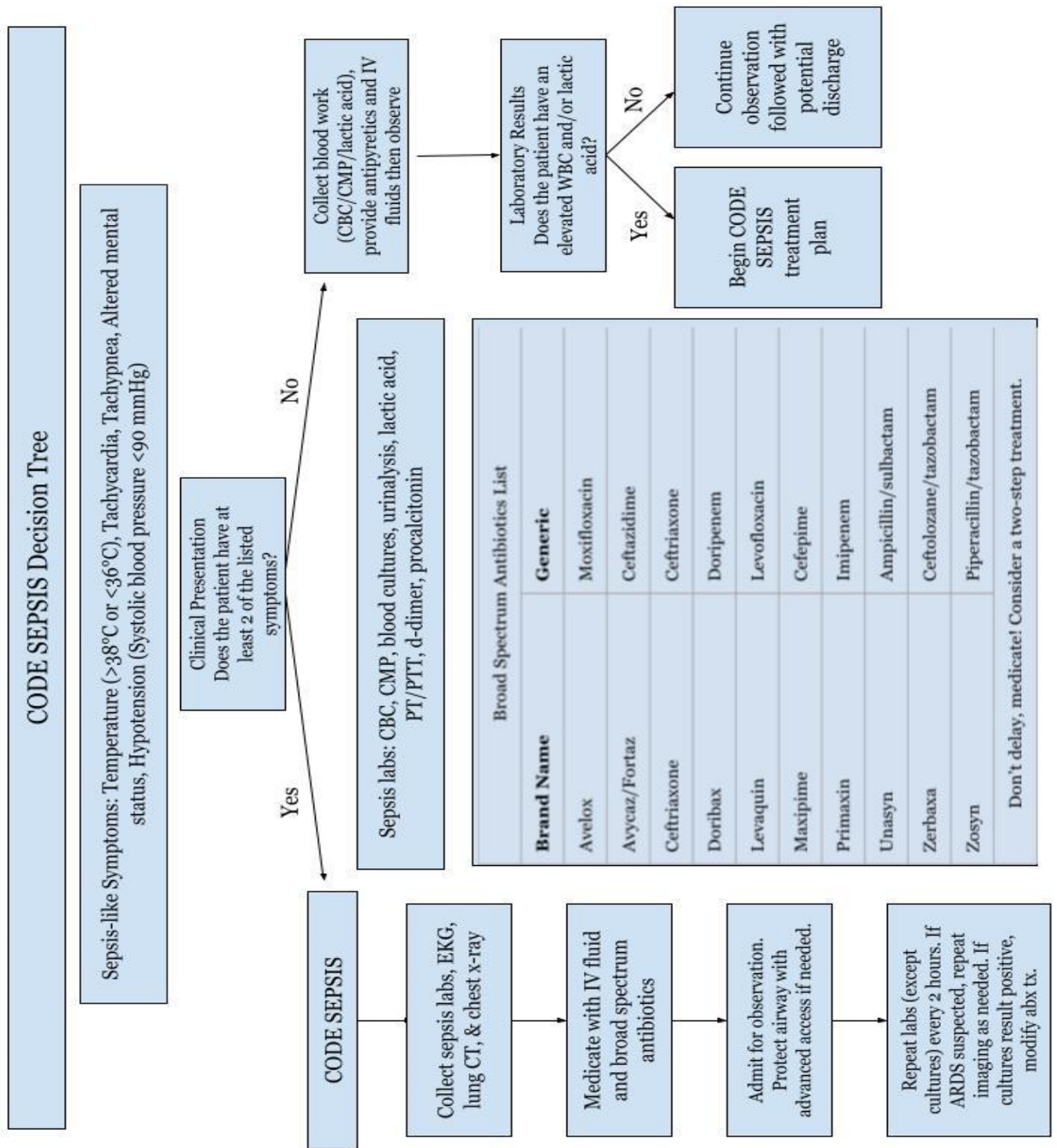
When cellular forms of therapies are exhausted, medicinal options do exist, but the path is not so clear. Sepsis-induced ARDS originates from massive inflammation caused by bacterial infections. An array of cytokines are produced, activating their respective receptors. Certain medications target specific chemokine/cytokine signaling pathways decreasing the vast immune response to allow epithelial repair. Anti-inflammatories, such as corticosteroids and prostaglandin, and vasodilators (nitric oxide and prostacyclin) work to inhibit the production of proinflammatory cytokines and reduce the adhesion of neutrophils to the alveolar membrane that could lead to invasion (8). The issue behind using multiple medications to allow healing of the lungs is the effect of each medication on an immune response or another medicine. Innate immunity responds to pathogen infections with immune cell recruitment and cytokine production. Utilizing anti-inflammatories represses the innate response to a bacterial infection. Sepsis provokes lung damage by ARDS, thus elongating a systemic disease for lung repair contradicts the body's natural response. The use of vasodilators decreases pulmonary vascular resistance, improving arterial oxygenation (8). Survival rates do not change with this form of therapy and can cause the patient to progress into septic shock by opposing the mechanism of action of vasoconstrictors.

If carried out successfully, the experimental treatments on the animal models present encouraging results for patients with ARDS. Due to the unpredictability of the immune system's response to foreign cells or medications, multiple forms of therapies must exist that address the needs of individual patients. The risks of undergoing such therapies substantially increase with

already compromised patients, which stresses the need for more ARDS preventative measures for septic patients.

## VII. CONCLUSION

Prolonging the treatment of sepsis contributes to the progression and manifestation of ARDS. Foreign pathogens recognized by the innate immune system trigger an adaptive response that ultimately disrupts the homeostasis of alveolar units. Without early treatment, pulmonary edema and fibrosis limit lung compliance, comprising the respiratory system. In detailing these changes at a cellular level, providers can aggressively treat sepsis and prevent ARDS progression. Treatment effectiveness against ARDS can be measured by collecting lung CT scans and procalcitonin levels. Initiating a broad-spectrum treatment plan and curtailing the plan to treat the specific pathogen in question sets the patient on a path to a good outcome. Although survival rates are low in progressed cases, studies in reconstructing alveolar units using stem cells show hopeful evidence. No set therapy exists; however, recent events, like the COVID-19 pandemic, exemplify the need for further investigation into this topic. This research serves as a stepping stone to treating sepsis and ARDS in emergency medicine and critical care settings.



**Figure 3. CODE SEPSIS Decision Tree.** Shown is a diagram listing the steps a provider should take depending on the patient’s clinical presentation. The figure shows a chart with possible broad-spectrum antibiotics and blood test orders.

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